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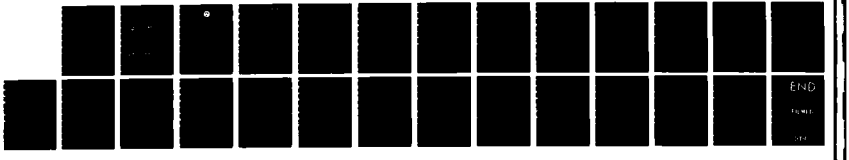

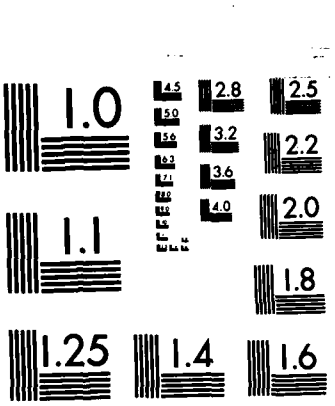

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ECOLOGICAL EVALUATION OF ORGANOTIN-CONTAMINATED SEDIMENT

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Naval Ocean Systems Center
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ADMINISTRATIVE INFORMATION

The work described herein was performed by the Marine Environment Branch, Code 522, Naval Ocean Systems Center, for the Naval Facilities Engineering Command.

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A standard dredged material bioassay was conducted with high levels of organotins to assess the toxicity and bioavailability of organotins associated with sediment and to determine if this sediment would qualify for ocean disposal. This study concluded that high levels of organotins in sediments do not a priori indicate a significant adverse impact on the marine environment after ocean disposal. The sediment tested would qualify for ocean disposal under the present guidelines administered by the Environmental Protection Agency and the Army Corps of Engineers.

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SUMMARY

OBJECTIVES

A standard dredged material bioassay was conducted on sediment with high levels of organotins to (1) assess the toxicity and bioavailability of organotins associated with sediment and (2) to determine if this sediment would qualify for ocean disposal. Since the Navy is considering Fleetwide implementation of organotin coatings and some commercial fleets are currently using them, the effects of organotins on the dredging permit process must be evaluated.

METHODS

Particulate-phase tests were conducted with Acanthomysis sculpta (mysid), Citharichthys stigmaeus (flatfish), and Acartia tonsa (copepod). Solid-phase tests were conducted with A. sculpta, Macoma nasuta (clam), and Neanthes arenaceodentata (polychaete worm). The bioassay also included an estimate of the potential for bioaccumulation of cadmium, chromium, copper, mercury, silver, pesticides, PCBs, petroleum hydrocarbons, and organotins.

RESULTS

The concentration of bis(tri-n-butyltin) oxide (TBTO) in sediment collected from the Commercial Basin, San Diego Bay, was 780 ppb. Initial concentrations of TBTO in particulate-phase test water was 0.49 ppb and in solid-phase test water was 0.20 ppb. Butyltins measured in test water and test sediments demonstrated that monobutyl-, dibutyl-, and tributyltin were all leached off Commercial Basin sediment. Treatment clams accumulated organotins to a concentration an order of magnitude above control clams (2.82 ppm TBTO vs 0.26 ppm TBTO) and a factor of four above treatment sediment.

CONCLUSIONS

Survival was high in all particulate-phase and solid-phase tests. There were no statistically significant differences in survival when controls were compared to treatments. As demonstrated for other contaminants in other studies, high levels of organotins in sediments do not a priori indicate a significant adverse impact on the marine environment after ocean disposal. The sediment tested would qualify for ocean disposal under the present guidelines administered by the Environmental Protection Agency and the Army Corps of Engineers.

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INTRODUCTION

The Commercial Basin in San Diego Bay is heavily used by both commercial and private vessels. These vessels are coated with antifouling paints that leach copper or organotins into the water column. A significant portion of these contaminants are adsorbed by the sediment. Previous measurements in the area have shown elevated copper levels in the water column, presumably from the antifouling coatings (Krett, 1979; Zirino et al., 1978). More recent analyses performed by the Naval Ocean Systems Center (NOSC) indicate the Commercial Basin water column has the highest levels of tributyltins as bis(tri-n-butyltin) oxide (TBTO) found in San Diego Bay (Valkirs et al., 1984). TBTO concentrations for water collected near the bottom ranged from 0.11 to 0.55 ppb. These water column values are close to those causing effects on sensitive marine organisms like mysids and copepods in laboratory tests (Salazar & Salazar, in preparation; Seligman, 1984; U'Ren, 1983). Corresponding sediment samples from the same area ranged from 32- to 560-ppb TBTO. This represents a concentration factor of approximately three orders of magnitude between the water column and the sediment.

We estimate more than 20 percent of the vessels in the Commercial Basin currently use organotin-based antifouling coatings. The environmental impact of organotins from these coatings is of concern to the Navy due to projected Fleet implementation of organotin-based antifouling coatings. As part of a research program on the fate and effects of organotins in the marine environment and a program to expedite Navy dredging, we performed a bioassay on sediment collected from Commercial Basin. This site was selected, even though it was not a Naval facility, because of the high concentration of TBTO found there. The purpose of this work was to (1) assess the toxicity and bioavailability of organotins associated with sediments and (2) determine if sediment with these high organotin loads would qualify for ocean disposal under the present guidelines.

According to Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972, sediments to be dumped into ocean waters must be evaluated to determine the potential environmental impact. Evaluations must be in accordance with criteria in the Federal Register, Vol. 42, No. 7, 11 January 1977 (EPA, 1977). Previously, evaluations were based only on chemical analyses of dredged material. Now, sediment chemical analyses are not required and bioassays are the primary basis for determining the potential for environmental impact. The intent of the law is to prevent an adverse environmental impact from ocean disposal of dredged material.

The standard bioassay test procedures have been published by the Environmental Protection Agency (EPA) and the Army Corps of Engineers (COE) in an implementation manual (EPA/COE, 1977). We conducted an ecological evaluation of Commercial Basin sediment according to the bioassay procedures outlined in this manual, even though no dredging or ocean disposal is planned for this sediment. This report presents the results of the evaluation performed on sediment from Commercial Basin, San Diego Bay.

METHODS

The procedures given in the implementation manual (EPA/COE, 1977) were followed wherever possible and practicable for most of the bioassay. Remember the manual is only a guide and need not be followed exactly. In 1983 the COE, Los Angeles District, outlined requirements and guidance in District Bioassay Testing Procedures. These regionally specific procedures were also followed wherever feasible. The most significant procedural modification used in these bioassays was a conceptual one. Instead of collecting sediment from three sites within each dredging area and conducting three bioassays per site as the manual suggests, we collected sediment from three different sites in the test area and pooled them for one bioassay. Sediment samples were pooled by mixing during collection, preparing particulate-phase slurries, and pre-sieving for the solid phase.

Although the manual suggests using 10-, 50-, and 100-percent test solutions of liquid and particulate phases, for this bioassay we used only the 100-percent particulate-phase solutions for two reasons. First, the suspended-particulate phase incorporates both a liquid and a particulate phase. Second, in all our previous bioassays we have not had a significant effect with the 100-percent particulate-phase solutions. Therefore, the lesser dilutions (10 and 50 percent) are superfluous. For these reasons, only 100-percent suspended-particulate phase solutions were prepared and tested. To increase statistical reliability, we used five replicates instead of the recommended three. EPA and COE agreed with these changes.

SEDIMENT

Collection Procedures

Three samples were collected along a pier on the northwest shore in Commercial Basin (Figure 1). Test sediment was collected from the upper 20-30 cm of bottom material by scuba divers using plastic buckets. On the surface, the sediment was dumped into 60-liter ice chests lined with large plastic bags to reduce potential contamination. Ice chests were used for ease in handling sediment and to maintain the temperature of sediment between collection and storage. The sediment was stored at 4 °C and used 48 hours after collection. Approximately 200 liters of reference/control and 100 liters of treatment sediment were collected.

Sediment Characteristics

Sediment that would serve as both a reference and a control was collected in an uncontaminated area off North Island near the mouth of the bay (Figure 1). Sediment from this area has been used as a combined reference/control in our 19 previous dredged material bioassays on San Diego Bay sediment. It can be used as a reference because mean grain size and composition are similar to disposal-site sediment. Available information suggests that the average grain size at the nearest interim EPA disposal site (LA-5) is between 0.030 mm (Emery & Butcher, 1952) and 0.079 mm (Marine Bioassay Laboratories, 1982).

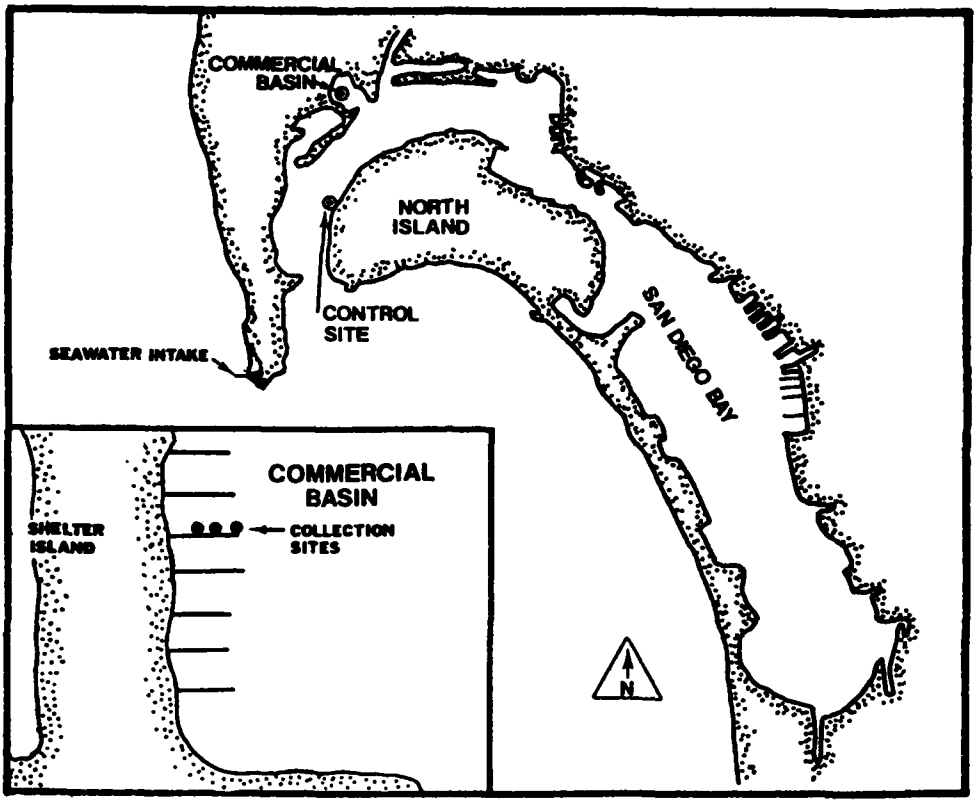

Figure 1. Location of Commercial Basin and control sediment collection sites in San Diego Bay and the seawater intake.

The mean grain size of the control sediment used in this bioassay was 0.0981 mm. Control sediment was composed of approximately 81-percent sand and 19-percent silt and clay, and was classified as fine sand (Wentworth, 1922). This was similar to disposal-site sediment composition, estimated at 67-percent sand and 33-percent silt and clay (Marine Bioassay Laboratories, 1982). Grain sizes for control and treatment sediments were measured by the tube drop method.

This sediment can also serve as a control because of its relatively low contamination. As part of our previous bioassays, we measured five heavy metals (Cd, Cr, Cu, Hg, and Ag), petroleum hydrocarbons, polychlorinated biphenals (PCBs), and pesticides in test sediments. Control sediment has almost always been significantly lower in these contaminants than treatment sediment (Salazar & Salazar, a, b, & c, in preparation; Salazar & U-Ren, a & b, in preparation; Salazar, et. al., 1980). In many cases, contamination was lower by approximately one order of magnitude. Relatively low contamination combined with sediment composition and grain size, similar to disposal-site sediment, justify using North Island sediment as a control. For expediency, this reference/control sediment will be referred to as control sediment.

SEAWATER

The manual suggests that disposal-site water be used if possible. This was impracticable because of the large volume required and the distance to the site. Near-shore seawater available at the NOSC Marine Sciences Laboratory was used for this bioassay. The seawater intake for this system is approximately 250 meters from shore (Figure 1). This natural seawater was passed through large sand filters prior to delivery to the laboratory.

Periodic water analyses have shown that the five heavy metals (Cd, Cr, Cu, Hg, and Ag) we routinely measure to assess bioaccumulation are present in only trace amounts. These concentrations are similar to those found in open-ocean water (Berhard & Zattera, 1975). Analyses of tissues from test animals in previous bioassays have shown no significant accumulation of heavy metals, hydrocarbons, PCBs, or pesticides (Salazar & Salazar, a, b, & c, in preparation; Salazar & U'Ren, a & b, in preparation; and Salazar et al., 1980).

PREPARATION OF TEST TANKS

Suspended-Particulate Phase

Test slurries were prepared in a 4:1 ratio, as the manual recommends, by adding 120 liters of control water to 30 liters of treatment sediment. Clean, aged 50-gallon plastic trash cans were used for mixing. The slurries were mixed by vigorous bubbling with compressed air at a rate of 50 liters per minute. After 30 minutes of bubbling, the air stones were removed and the slurries were allowed to settle for 1 hour. After settling, the supernatant was pumped directly into test tanks for the 100-percent suspended-particulate phase test.

Solid Phase

Treatment and control sediments were sieved through a 1.0-mm stainless steel screen to remove endemic organisms. Solid-phase test tanks were prepared with a 15-mm layer of treatment sediment on top of a 30-mm layer of control sediment. Control tanks for the solid-phase tests were filled with control sediment to a depth of 45 mm. After distribution of the sediments, the tanks were slowly filled with seawater. Animals were added after preparation of the solid-phase tanks.

TEST ANIMALS

Suspended-Particulate Phase

For the particulate phase, the manual suggests using phytoplankton or zooplankton, a crustacean or mollusk, and a fish. A copepod (planktonic crustacean), a mysid (hypoplanktonic crustacean), and a fish were used in this phase of the sediment bioassay. The manual further suggests that organisms from the disposal site be used where possible. It was not practicable to use species collected from the disposal site because they were neither well-known nor easily obtained.

Acartia tonsa, a copepod, was selected to satisfy the zooplankton requirement in the particulate phase. Acanthomysis sculpta was used as the representative crustacean species. The speckled sanddab, Citharichthys stigmaeus, was the third species used for the particulate-phase tests. All C. stigmaeus suriving at the end of the 96-hour test were weighed and measured. These animals had a mean length of 62.8 mm and a mean wet weight of 2.75 gm.

Solid Phase

The manual suggests using three species for solid-phase tests: one filter-feeder, one deposit-feeder, and one borrowing species. We further suggested that the species be selected to include a crustacean, an infaunal bivalve, and an infaunal polychaete. As with the particulate phase, it was not practicable to use species from the disposal site. Solid-phase tests for the Commercial Basin bioassay were conducted with A. sculpta (mysids), Neanthes arenaceodentata (polychaete worms), and Macoma nasuta (clams).

Estimates of lengths and weights for clams were made by measuring and weighing 50 animals per treatment at the end of the bioassay. Test animals had a mean length of 40.6 mm and a mean wet weight of 8.24 gm.

Bioaccumulation

The manual suggests that all biological evaluations of the dredged material include an assessment of the potential for contaminants to be bioaccumulated in the tissues of marine organisms. We used M. nasuta as one bioaccumulator because of the relatively large amount of tissue available for chemical analysis. We used N. arenaceodentata as the second required bioaccumulator.

Animal Collection

We collected copepods and mysids just outside the mouth of San Diego Bay on the first day of the test. Copepods were collected by slowly towing a net (175-micron mesh) in open water just beneath the surface. Mysids were collected in the surface canopy of the kelp with buckets. Temperature upon arrival was 18 °C for both species.

Three of the five species of marine organisms used in these bioassays were purchased from two commercial suppliers. Fish and clams were collected by Brezina and Associates, Dillon Beach, California. The fish were collected near the mouth of Tomales Bay, California, in 10-15 feet of water. The clams were collected in an intertidal mudflat, also in Tomales Bay.

N. arenaceodentata is kept in continuous culture by Dr. Donald Reish, California State University, Long Beach. The worms were obtained 1 day prior to the start of the test.

TEST CONDITIONS

Polycarbonate tanks, which exhibit low adsorption of trace metals and organotins, were used as test containers in the particulate-phase and solid-phase tests for all species except copepods. We used Pyrex beakers for copepods because Pyrex glass is also nonadsorptive and the size is convenient.

Temperature at the 600-foot-deep disposal site probably ranges between 8 and 10 °C. We used water temperatures between 13.5 and 16.0 °C for this bioassay because they are closer to the optimum for maintaining these particular test animals and the limits of natural ocean temperature and the flowthrough seawater system. Physical-chemical parameters were measured daily. Cool white fluorescent bulbs were used to approximate the spectral output of the sun, but irradiance in the test containers was significantly less than the 1200 microwatts/cm^2 recommended by the manual. Irradiance was measured with a photometer. For each test species, the values obtained (microwatts/cm^2) were as follows: C. stigmaeus (100-500); N. arenaceodentata (1-250); A. sculpta (1-250); M. nasuta (100-250); and A. tonsa (5-50). The light regime consisted of a 14L:10D cycle.

Suspended-Particulate Phase

The copepods were held in 400-ml Pyrex beakers, mysids in 4-liter polycarbonate tanks, and fish in 16-liter polycarbonate tanks. All particulate-phase tests were conducted under static conditions for 96 hours. Five replicates were used for each treatment and control condition with 10 organisms in each replicate. The fish were vigorously aerated at a rate between 500 and 1300 ml/minute. Mysids were moderately aerated at a rate of 3-7 ml/minute. Copepods were not aerated.

Copepods were fed maintenance levels of the phytoplankton Isochrysis galbana on days 1, 2, and 3 of the bioassay (1,000 - 10,000 cells/copepod/day). Mysids were fed maintenance levels of brine shrimp nauplii (25-30 nauplii/mysid/day). The fish were not fed during the bioassay.

Live animals were counted at the end of each test. It was impractical to count live copepods and mysids every day, as suggested in the manual, since the only reliable method involves removing the animals. This procedure would severely stress the delicate test organisms and could adversely affect the results. Fish were counted daily and dead animals removed. During the particulate-phase tests, seawater temperature was maintained at 13.5-15.3 °C, salinity between 31.0 and 33.0 ppt, dissolved oxygen between 6.0 and 8.0 ppm, and pH between 7.4 and 7.8 for all species tested.

Solid Phase

The solid-phase tests were run for 10 and 20 days. The 10-day tests utilized A. sculpta only. The mysids were maintained in 4-liter polycarbonate tanks under static conditions. Five replicates were prepared for each treatment and control condition with 20 organisms in each replicate. As in the particulate phase tests, mysids were gently aerated (3-7 ml/minute) and fed brine shrimp nauplii daily (25-33 nauplii/mysid/day).

Clams (M. nasuta) and worms (N. arenaceodentata) were held in test sediments for 20 days with flowthrough seawater. These organisms fulfilled requirements for both the 20-day survival portion and the bioaccumulation portion of the bioassay. Clams and worms were maintained in separate 16-liter polycarbonate tanks. Five replicates were prepared for each treatment and control condition with 20 clams per replicate and 25 worms per replicate.

Clams were not fed. Worms were fed TetraMin SM80 fish food on days 4, 7, 11, 14, and 18. Fish food flakes were ground with a mortar and pestle and combined with seawater to create a slurry that was injected into the water with a large syringe. Each tank received 125 mg of food (5.0 mg/worm) on feeding days. The number of live animals was determined at the end of the test instead of during the test, as the manual recommends, because counting during the test disturbs the sediment regime and places additional stress on test animals.

Seawater was delivered to these tanks by means of a manifold system developed as part of the Navy program to evaluate the toxicity of organotin antifouling compounds (Meador et al., 1984). Flow rates were approximately 170 ml/minute. All tanks were aerated at a rate between 500 and 1000 ml/minute. For these animals, seawater temperature was maintained at 14.0-16.3 °C, salinity at 31.0-34.0 ppt, dissolved oxygen at 6.2-8.3 ppm, and pH at 7.6-8.0.

Bioaccumulation

At the end of the bioassay, test sediment was removed by wet-sieving. Clams and worms were counted and immediately returned to their original flow-through test containers. The clams and worms were then fed rations of TetraMin; worms were fed 5.0 mg each and clams 14.0 mg each. These animals were held for a 24-hour depuration period. The animals were fed to encourage elimination of ingested sediment. After the 24-hour depuration period, the animals were removed and tissues were frozen for subsequent bioaccumulation estimates.

Tissues and sediment samples were analyzed for cadmium, chromium, copper, mercury, silver, pesticides, PCBs, petroleum hydrocarbons, and organotins. Cadmium, chromium, copper, and silver were analyzed by graphite furnace atomic absorption spectroscopy; mercury was analyzed by cold vapor atomic absorption spectroscopy. Pesticides, PCBs, and petroleum hydrocarbons were measured by gas chromatography. The manual does not require chemical analysis of sediment. It was analyzed here to document the amount of contaminants available for bioaccumulation in control and treatment sediments and to confirm collection of typical control to sediment selection. Analytical measurements were conducted by Anatech Laboratories, Santa Rosa, California.

All organotin measurements were made at NOSC. To determine the amount of organotin released from sediment upon mixing, seawater samples were taken from the suspended particulate-phase preparation container just before adding the test slurry to treatment tanks. Solid-phase water samples were taken from mysid tanks on the first and last days of the bioassay. Butyltin species were measured in these and control water samples using a hydride reduction method followed by hydrogen flame atomic absorption spectroscopy (Valkirs et al., 1984).

The total tin concentration in test sediments was measured at the beginning and end of the bioassay. Bulk samples from the collection chests (Control and Commercial Basin) and test samples from solid-phase mysid and clam tanks were taken on the first day of the bioassay. Samples were also taken from solid-phase control, mysid, worm, and clam tanks on the last day of

the test. The concentration of total solvent-extractable tin was measured by graphite furnace atomic absorption spectroscopy (Seligman, 1984).

Additional clams from the 20-day bioaccumulation study were used to determine organotin uptake. Accumulated organotins were extracted from ground clam tissues with methylene chloride. Samples were placed on a rotary shaker for 24 hours to ensure complete extraction. The concentration of total solvent-extractable organotin was determined by graphite furnace atomic absorption spectroscopy. The tin values expressed as μg Sn/gm dry tissue were multiplied by 2.5 to estimate TBTO concentration and then multiplied by 0.20 to estimate wet-weight concentration comparable to our other bioaccumulation data.

DATA ANALYSIS PROCEDURES

Statistical methods outlined in the manual were followed wherever possible. Homogeneity of variances in bioaccumulation data was assessed with the Max-F Test. Significant differences were assessed by either the Student's T-Test if the variances were homogeneous or the Mann-Whitney U-Test if they were not.

Survival data for control and treatment organisms in the particulate- and solid-phase tests are discrete data. Therefore, instead of the t-test as recommended by the manual, we used its nonparametric equivalent, the Mann-Whitney U-Test (Dixon & Massey, 1969; Zar, 1974) to compare treatment and control survival. Significant results for all statistical tests were determined by the critical values ($a = 0.05$) of the appropriate distributions.

RESULTS

The Commercial Basin bioassay was conducted in March 1984. Survival percentages for the particulate-phase and solid-phase tests are presented in Tables 1 and 2. Results of the statistical analyses are presented in Table 3. No statistically significant differences in survival were found between controls and treatments for any of the species tested. The concentration of contaminants measured in test animal tissues and in test sediments are presented in Table 4. The means for these data and the results of statistical analyses are included in Table 5. No statistically significant differences in bioaccumulation were found between control and treatment groups except for copper, PCBs, and organotins in *M. nasuta* and copper and silver in *N. arenaceodentata*. The concentrations of organotins in test sediments, test water, and clam tissues are given in Table 6.

Suspended-Particulate Phase

In the particulate-phase tests with fish, control survival was 98 percent and treatment survival was 96 percent. Control and treatment survival for mysids was 100 percent. For copepods, control survival was 88 percent and treatment survival was 82 percent. There were no statistically significant differences in survival between control groups and the Commercial Basin treatment groups for any of the animals tested.

Table 1. The number of fish (Citharichthys stigmaeus), mysids (Acanthomysi sculpta), and copepods (Acartia tonsa) surviving after 96 hours of exposure to 100-percent particulate-phase material.

	Replicate Number	Control	Commercial Basin
Citharichthys stigmaeus	1	9	10
	2	10	10
	3	10	10
	4	10	8
	5	10	10
		98%	96%
Acanthomysis sculpta	1	10	10
	2	10	10
	3	10	10
	4	10	10
	5	10	10
		100%	100%
Acartia tonsa	1	6	8
	2	8	8
	3	10	8
	4	10	8
	5	10	9
		88%	82%

Table 2. The number of mysids (Acanthomysis sculpta), worms (Neanthes arenaceodentata), and clams (Macoma nasuta) surviving the solid-phase tests. Mysids were exposed for 10 days; clams and worms for 20 days.

	Replicate Number	Control	Commercial Basin
Acanthomysis sculpta (10-Day test)	1	18	16
	2	17	19
	3	20	17
	4	20	19
	5	20	15
		95%	86%
Neanthes arenaceodentata (20-Day test)	1	23	20
	2	25	21
	3	24	20
	4	15	23
	5	19	25
		84.8%	87.2%
Macoma nasuta (20-Day test)	1	20	19
	2	20	20
	3	20	20
	4	20	20
	5	19	20
		99%	99%

Table 3. Statistical values for determining significant mortality in 100-percent particulate- and solid-phase tests for the Commercial Basin bioassay. (Mann-Whitney U-test, 95-percent CL). A statistically significant difference exists if the calculated value is greater than the critical.

	Mann-Whitney U-Test Results		
	Calculated	Critical	Decision
100 Percent Particulate Phase:			
Citharichthys stigmaeus	13.0	21.0	NSD
Acanthomysis sculpta	----	21.0	NSD*
Acartia tonsa	15.0	21.0	NSD
Solid Phase test:			
Acanthomysis sculpta	20.5	21.0	NSD
Neanthes arenaceodentata	13.0	21.0	NSD
Macoma nasuta	----	21.0	NSD*

NSD = No significant difference in mortality.
NSD* = No significant difference in mortality determined by inspection of data.

Table 4. The concentration (ppm) of trace metals, pesticides (TICH), petroleum hydrocarbons, and PCBs measured in the tissues of clams (Macoma nasuta), worms (Neanthes arenaceodentata), and in seawater and sediments. Data are expressed on wet-weight basis.

Macoma nasuta

Sample	Cadmium	Chromium	Copper	Mercury	Silver	PHF	PCBs	TICH
C-1	0.150	0.520	2.000	0.050	0.130	<0.100	0.010	<0.0020
C-2	0.150	0.410	1.700	0.060	0.130	<0.100	0.004	<0.0010
C-3	0.120	0.420	2.200	0.030	0.100	<0.100	0.010	<0.0020
C-4	0.130	0.390	2.300	0.020	0.130	<0.100	0.010	<0.0020
C-5	0.140	0.400	1.700	0.040	0.080	<0.150	0.010	<0.0030
Basin-1	0.120	0.340	2.800	0.060	0.120	<0.100	0.040	<0.0020
Basin-2	0.090	0.370	4.200	0.060	0.090	<0.100	0.050	<0.0030
Basin-3	0.130	0.290	2.900	0.040	0.130	<0.200	0.040	<0.0020
Basin-4	0.120	0.550	4.100	0.050	0.120	<0.100	0.040	<0.0020
Basin-5	0.070	0.330	2.600	0.060	0.090	<0.100	0.050	<0.0020

Neanthes arenaceodentata

Sample	Cadmium	Chromium	Copper	Mercury	Silver	PHF	PCBs	TICH
C-1	0.030	0.200	8.000	0.100	0.090	<1.500	0.090	<0.0020
C-2	0.020	0.100	6.000	0.100	0.070	<1.400	0.080	<0.0020
C-3	0.040	0.100	7.000	0.100	0.070	<1.400	0.080	<0.0020
C-4	0.050	0.300	5.000	0.200	0.050	<4.000	0.240	<0.0050
C-5	0.030	0.200	6.000	0.200	0.070	<1.800	0.100	<0.0020
Basin-1	0.040	0.200	17.000	0.100	0.130	<1.800	0.100	<0.0020
Basin-2	0.030	0.400	20.000	0.100	0.140	<2.000	0.130	<0.0030
Basin-3	0.030	0.200	10.000	0.100	0.080	<2.000	0.100	<0.0020
Basin-4	0.030	0.200	18.000	0.100	0.110	<1.600	0.090	<0.0020
Basin-5	0.030	0.200	18.000	0.100	0.120	<1.700	0.100	<0.0020

Seawater and Sediment Samples

Sample	Cadmium	Chromium	Copper	Mercury	Silver	PHF	PCBs	TICH
Water	0.001	0.011	0.002	0.0005	0.002	<0.004	0.0002	<0.00005
Control	0.700	15.000	16.000	0.0980	0.800	<0.100	0.006	<0.0010
Basin	0.900	26.000	210.000	2.7000	0.800	<0.100	0.025	<0.0010

C = Control
PHF = Petroleum hydrocarbon fraction.
PCB = Polychlorinated biphenyls quantitated as Aroclor 1254.
TICH = Total identifiable chlorinated hydrocarbon pesticides.

Table 5. Mean contaminant concentrations (ppm) and statistical values used for determining bioaccumulation potential in the Commercial Basin sediment.

Macoma nasuta

Contaminant	Control	Test	Common	Calc.	Crit.	Decision
Cadmium	0.138	0.106	yes	T: 2.53	2.306	SD**
Chromium	0.428	0.376	yes	T: 1.02	2.306	NSD
Copper	1.980	3.320	yes	T: 3.68	2.306	SD
Mercury	0.040	0.054	yes	T: 1.72	2.306	NSD
Silver	0.114	0.110	yes	T: 0.30	2.306	NSD
Pesticides	0.002	0.002	yes	T: 0.53	2.306	NSD
PCBs	0.008	0.044	yes	T:12.90	2.306	SD
Pet. Hydro.	0.110	0.120	yes	T: 0.45	2.306	NSD

Neanthes arenaceodentata

Contaminant	Control	Test	Common	Calc.	Crit.	Decision
Cadmium	0.034	0.032	yes	T: 0.37	2.306	NSD
Chromium	0.180	0.240	yes	T: 1.10	2.306	NSD
Copper	6.400	16.000	no	U:25.00	21.000	SD
Mercury	0.140	0.100	no	U:17.50	21.000	NSD
Silver	0.070	0.116	yes	T: 3.81	2.306	SD
Pesticides	0.026	0.022	yes	T: 0.63	2.306	NSD
PCBs	0.118	0.104	no	U:17.00	21.000	NSD
Pet. Hydro.	2.020	1.820	no	U:17.50	21.000	NSD

NSD = No significant difference.
SD = Significant difference.
SD** = Significantly lower concentration in test sample.
T = Student's T-Test.
U = Mann-Whitney U-Test.

Table 6. Concentration (ppb) of monobutyltin (MBTO), dibutyltin (DBTO), and tributyltin (TBTD) measured in the sediments and seawater from the Commercial Basin bioassay. TBTO was measured in Macoma nasuta tissues, and estimates of bioaccumulation were made on a wet weight basis of μg Sn/gm tissue.

Seawater

	Initial			Final		
	MBTO	DBTO	TBTO	MBTO	DBTO	TBTO
Control Seawater	ND	ND	ND	ND	ND	ND
Particulate-Phase Seawater	0.10	0.30	0.49	NS	NS	NS
Solid-Phase Mysid Tank Water	0.67	2.00	0.20	0.03	0.05	0.25

Sediments

	Initial	Final
	TBTO	TBTO
Control	62.50	72.50
Bulk Commercial Basin	780.00	NS
Solid Phase - Mysid Tanks	318.00	155.00
Solid Phase - Clam Tanks	610.00	267.50
Solid Phase - Worm Tank	NS	545.00

Clam Tissues

	TBTO
Control	0.26
Commercial Basin	2.82

Statistical Analysis of TBTO in Clam Tissue

Contaminant	Control	Test	Common	Calc.	Crit.	Decision
TBTO	0.26	2.82	no	U:16	U:15	SD

NS = Not sampled.
ND = Not detectable.
SD = Significant difference.

Solid Phase, 10-Day Tests

In 10-day solid-phase tests with mysids, control survival was 95 percent and treatment survival was 86 percent. There was no statistically significant difference in survival between controls and treatments.

Solid Phase, 20-Day Tests

In 20-day solid-phase tests with clams, both control and treatment survival was 99 percent. In 20-day solid phase tests with worms, control survival was 84.8 percent and treatment survival was 87.2 percent. There was no statistically significant difference in survival between controls and treatments for either species.

Bioaccumulation

There was a significant accumulation of copper and silver in the tissues of N. arenaceodentata and a significant accumulation of copper, PCBs, and organotin in the tissues of M. nasuta. There was no significant accumulation of other measured contaminants in the tissues of N. arenaceodentata or M. nasuta when controls were compared with treatments for the Commercial Basin bioassay. The significance of the silver accumulation is questionable as the amounts determined in control and treatment sediments were similar (0.800 ppm).

The total amount of organotins accumulated in clam tissues is shcwn in Table 6. Treatments accumulated significantly more tin than controls. Control clam tissues were measured to contain 0.26-ppm organotin as TBTO. Clams exposed to the Commercial Basin sediment accumulated 2.82-ppm organotin as TBTO. These values were determined on a wet-weight basis and demonstrate that organotins associated with sediments are bioavailable.

Organotin Measurements

The concentration of butyltin species in particulate-phase water was measured as 0.10-ppb monobutyltin, 0.30-ppb dibutyltin, and 0.49-ppb tributyltin. In solid-phase water from mysid tanks, the initial concentration of tributyltin was 0.20 ppb, about half that of particulate-phase water. However, both monobutyltins and dibutyltins were higher at 0.67 and 2.00 ppb, respectively. At the end of the solid-phase mysid test, water from these tanks was measured at 0.03-ppb monobutyltin, 0.05-ppb dibutyltin, and 0.25-ppb tributyltin (Table 6). Organotins were not detectable in the near-shore seawater obtained through the NOSC seawater system.

The concentration of solvent-extractable tin (as TBTO) in control sediment was between 62.50 and 72.50 ppb. The bulk samples of Commercial Basin sediment (from the collection chests) contained 780.0-ppb tin as TBTO. The concentration of tin in Commercial Basin sediment from test tanks was between 318.0 and 610.0 ppb as TBTO. This confirms that the amount of tin in the treatment sediment was relatively high and in the control sediment was relatively low. The amount of tin remaining in the sediment from the solid-phase mysid, clam, and worm tanks at the end of the bioassay was 155.0, 267.5, and 545.0 ppb as TBTO, respectively (Table 6). This decrease in TBTO concentration demonstrates that tins had leached off the treatment sediment.

The composition of control sediment is much different than Commercial Basin sediment. In previous measurements of sediments from another control site near the entrance to San Diego Bay, tributyltins were below the levels of detection (Seligman, 1984). This suggests that most of the tin found in our control sediment is not TBTO, but other organically bound tins. Most of the tin in the Commercial Basin sediment is probably tributyltin and its degradation products. Seligman (1984) showed that sediment from the Commercial Basin collection site contains 77-percent tributyltin. To simplify the discussion, the concentration of organically extractable tin multiplied by 2.5 will be used as a relative TBTO concentration.

Sediment Characteristics

The mean grain size of the control sediment was 0.0981 mm. The composition was 81.0-percent sand with 10.6-percent silt and 8.4-percent clay. This was classified as fine sand. The mean grain size of Commercial Basin sediment was 0.0302 mm with a composition of 34.3-percent sand, 5.14-percent silt, and 14.3-percent clay. This sediment was classified as sandy-silt (Wentworth, 1922).

DISCUSSION

A standard dredged material bioassay was conducted on sediment contaminated with organotins collected from Commercial Basin in San Diego Bay. The purpose of this study was to assess the toxicity and bioavailability of organotins associated with sediment and to determine if this organotin-contaminated sediment would qualify for ocean disposal. On the basis of organotin field measurements of Commercial Basin sediment and water and concentrations known to cause effects on sensitive planktonic organisms in the laboratory, we anticipated this would be the first sediment from San Diego Bay to produce significant mortalities in sensitive species from a standard sediment bioassay. As part of a Navy program to study the fate and effects of organotins in the marine environment, sediments have been measured in a number of harbors. Measurements in San Diego Bay showed that Commercial Basin sediment and water had the highest concentrations of organotin. Sediment values ranged from 32- to 560-ppb TBTO. Surface water samples from Commercial Basin were measured between 0.01- and 0.18-ppb TBTO, while bottom water samples varied from 0.11- to 0.55-ppb TBTO (Valkirs et al., 1984).

A 96-hour LC_{50} of 1.0-ppb TBTO was estimated for A. tonsa with a static-renewal toxicity test. In this same study, a 144-hour effective concentration, or that concentration causing some effect on 50 percent of the animals after 144 hours, was calculated to be 0.4-ppb TBTO (U'Ren, 1983). Our previous work on the mysid Metamysidopsis elongata has estimated a 96-hour LC_{50} between 0.5- and 0.9-ppb TBTO for juvenile animals. For adults the LC_{50} was estimated to be about 3.0 ppb-TBTO (Seligman, 1984). In addition, Salazar and Salazar (1981) determined that equivalent TBTO concentrations prepared from stock chemical solutions and leachates from antifouling coatings had the same effect on mysids. Recent work at NOSC has estimated a 96-hour LC_{50} of 0.6-ppb TBTO for juvenile A. sculpta mysids and a 96-hour LC_{50} of 1.7-ppb TBTO for

adult A. sculpta mysids. All of these factors led to the selection of Commercial Basin sediment as an example of organotin contamination that might cause an effect on sensitive bioassay animals. A standard dredged material bioassay was used to predict the effects of organotins on bioassays required for future dredging permits.

The measured concentration of TBTO in Commercial Basin sediment from treatment tanks varied from 318- to 610-ppb extractable butyltin and confirmed that we had collected contaminated sediment. These concentrations are lower than concentrations measured for bulk sediment samples from the collection chests (780-ppb TBTO) because treatment sediment was actually collected from test tanks and may have included some control sediment from the bottom layer of the tank. The concentration of TBTO in treatment sediment is approximately 5 to 10 times higher than in our control sediment. These high TBTO values are similar to our previous measurements of Commercial Basin sediment.

Measurements of the water and sediment in solid-phase and particulate-phase test tanks indicate that organotins were leached from the test sediment. Although organotins were not detectable in control seawater, initial TBTO concentrations in mysid tanks measured on the first day of the test varied from 0.20 ppb in solid phase water to 0.49 ppb in particulate-phase water. The concentration of TBTO in test sediment decreased concomitantly during the test. These measurements confirmed that TBTO from Commercial Basin sediment was being released into test water. The speciated butyltin measurements demonstrated that the more soluble and less toxic monobutyltin and dibutyltin compounds were also released from the sediment.

Fish, clams, and worms all demonstrated high survival after continuous exposure to TBTO. These results were predictable based on their tolerance to other toxicants and results from previous bioassays. Due to their high sensitivity to organotins, significant mortalities in mysids and copepods exposed to organotin-contaminated sediments were expected, but were not observed. Control and treatment mysid survival was 100 percent in the 96-hour particulate-phase test, where TBTO concentrations were initially measured at 0.49 ppb. This was not expected. The estimated 96-hour LC_{50} for mysids was between 0.6- and 3.0-ppb TBTO and for copepods approximately 1.0-ppb TBTO. Possibly the initial concentration of TBTO decreased during the 96-hour test. However, mysids and copepods were exposed to concentrations of TBTO that approximated those that have previously produced significant effects. In the 10-day solid-phase test with mysids, treatment survival was 86 percent and was not significantly different than control survival of 95 percent. The concentration of TBTO varied between 0.20 and 0.25 ppb during the 10-day test. Copepods also did not exhibit an effect after TBTO exposure. Treatment survival was 82 percent for animals exposed to 0.49-ppb TBTO, while control survival was 88 percent.

Considering the rationale for replacing chemical analyses with bioassays for the regulation of dredged material disposal, perhaps these results should have been expected. The Corps of Engineers Dredged Material Research Program has shown that bulk metal analyses of test sediments do not correlate with metal bioavailability (Neff et al., 1978). Nathans and Bechtel (1977) have

further suggested that the magnitude of bioaccumulation does not always reflect sediment DDT concentration. Bioassay results give a normalized estimate for evaluating the potential for environmental impact after ocean disposal. Further, bioassays eliminate selecting which contaminants should be measured and which should be eliminated. This is particularly important when there is a chance of eliminating the one contaminant that could be the most significant. The bioavailability of contaminants in sediments is highly variable and dependent on a variety of factors. These include the physical properties of the sediments, chemical state of the contaminants, and the chemical and biological parameters of the water (Jennet et al., 1980).

The bioavailability of contaminants and their actual toxicity in seawater is also highly variable. Although a minimum concentration of certain trace metals is important for phytoplankton growth (Guillard & Ryther, 1962), the form or chemical state of those metals determines whether they are usable by organisms (Johnston, 1984). Studies on some species of phytoplankton show that growth inhibition and copper uptake are related to cupric ion activity and not to total copper concentration (Sunda & Guillard, 1976). Along with others, we have shown that toxicity to species used in this bioassay is also related to the state of the cupric ion tested (Salazar & Salazar, in preparation). In experiments with mysids and brine shrimp nauplii, tributyltin was far more toxic than its degradation products, dibutyltin and monobutyltin (Seligman, 1984). The addition of sediment to test tanks reduces the toxicity of copper to Neanthes arenaceodentata (Pesch & Morgan, 1978), while the type of sediment influences copper toxicity and copper bioaccumulation (Pesch, 1979).

Hence, the presence of sediment in toxicity tests will probably reduce toxicity and bioavailability of certain toxicants for a variety of animals. Since most organotin toxicity tests have been conducted without sediment, organotin toxicity has probably been overestimated. Toxic concentrations reported from those experiments are real but the abundance of naturally occurring organics in those tests was probably abnormally low. Thus, standard toxicity tests in glass aquaria without sediment do not realistically represent natural conditions. The addition of sediment, particulates, or other organic compounds provides a much better approximation of natural conditions in the marine environment. The expected toxicity of organotin-contaminated sediment from Commercial Basin was not realized because the organotins were probably in a form not readily available to the test animals.

Barber and Ryther (1969) and Johnston (1964) have shown that organic chelators are often the most crucial factor in bioavailability of nutrients for phytoplankton growth in seawater. Also, Morel and Morel-Laurens (1981) have suggested that, except for copper and iron, complexing agents have a relatively minor effect on initial complexation in seawater and that organic compounds adsorbed on the surface of sediment particles control the adsorption of metals. Although the actual chemical mechanism is unclear, some organics found in natural sediments can reduce toxicity and bioavailability. This study suggests that organics in Commercial Basin sediment reduce toxicity and bioavailability of organotins in that sediment.

In addition to naturally occurring organic chelators in sediments that reduce toxicity and bioavailability, animals also have the ability to sequester and detoxify contaminants. Since previous measurements of Commercial Basin sediment have shown elevated levels of copper and organotin, it is not surprising that M. nasuta bioaccumulated significant amounts of each of these contaminants. The environmental significance of the bioaccumulation estimate is unclear. Treatment clams were found to accumulate organotins to a concentration an order of magnitude above control clams (2.82-ppm TBTO compared to 0.26-ppm TBTO) and a factor of four above Commercial Basin sediment. Since the animals survived the experiment, these internal concentrations are apparently sublethal. Accumulated tins were probably modified by the animals to become biologically unavailable. Accumulated tins could have combined with lipids to reduce toxicity. They could have been bound to methallothioneins (Roesijadi, 1981) or sequestered within cellular organelles (George et al., 1978). In each case, there is a reduced opportunity for toxic expression.

Thus, when estimating the potential toxicity of organotin antifoulant leachates from Navy hulls to marine organisms, more effort should be directed toward duplicating natural conditions. This will prevent overestimating toxicity values and will reduce the significance of questionable values currently appearing in the literature. For our purposes, however, organotin-contaminated sediments from Commercial Basin, San Diego Bay, would qualify for ocean disposal under the present guidelines. Questions could be raised about the significant uptake of copper, the potential accumulation of organotins, or even the validity of the bioassay itself. With all its shortcomings, the bioassay as conducted here is probably the best available estimator of environmental impact after ocean disposal because it accounts for bioavailability. Changes could be made in species selection, test containers, test duration, etc.; but as a regulatory tool, it is probably all that could be expected at this time.

The other problem is that new contaminants like organotins are not well known. As they are studied further, new insight should be gained into improving the required ecological evaluation of dredged material. The Navy research programs to expedite dredging and study the fate and effects of organotins in the marine environment will help guide the assessment of the potential for environmental impact. However, as the use of organotins increases, bioassays should include an assessment of the potential for bioaccumulation of these compounds. Further, much additional work will be required to fully understand the chemistry and bioavailability of organotins and to predict their effect on the marine environment.

CONCLUSIONS

The results of this bioassay suggest that the sediment from Commercial Basin, San Diego Bay, should not have a significant impact on the marine environment if discharged into ocean waters. These results also suggest that organotins found in sediments at relatively high levels are not necessarily toxic to marine life. Probably the organotins present were not as bioavailable as originally anticipated, and the approach in testing organotin toxicity should be reevaluated in terms of environmental significance as well as adsorption on sediment and detoxification by animals.

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